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Phytolith Analysis on Dental Calculus, Enamel Surface, and Burial Soil: Information About Diet and Paleoenvironment

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ABSTRACT Silica phytoliths (microscopic remains originating in plant tissues) have been identified on the enamel surface and dental calculus of a sample of teeth selected from well preserved skeletons from a Late Roman necropolis in Tarragona (Spain). Phytoliths were observed by scanning electron microscopy (SEM) and their siliceous nature was confirmed by X-ray microanalysis. The phytoliths were compared to those of soil samples from both the areas of the tombs corresponding to the abdomen and the periphery of the skeletons, and were classified taxonomically by comparison with a large collection of silica particles from modern plants in the Mediterranean area. Most of the phytoliths identified on the enamel and the dental calculus belong to the family of Poaceae, while the phytoliths from the abdominal area belong to Poaceae, Leguminosae, Cyperaceae, and Chenopodiaceae. Results are concordant with archaeological, ecological, and historical data from the same site, and with the human Mediterranean diet. If done properly, the study of phytoliths can provide direct information about the vegetable diet of past human populations, and could be applied to the study of human fossils.

In the last few years, several researchers have focused on the study of the diet and feeding behavior of living and extinct primates through the microscopic examination of dental microwear (Puech, 1978, 1979; Ryan, 1979; Walker, 1979, 1981; Puech and Pant, 1980; Puech et al., 1980; Fine and Craig, 1981; Gordon, 1982; Grine, 1984, 1986; Teaford and Walker, 1984; Teaford, 1985; Walker and Teaford, 1988; Teaford and Robinson, 1989; Ryan and Johanson, 1989; Maas, 1991; Bullington, 1991; among others). Since most of the studies attempt to establish associations between specific food items and specific patterns of microwear in human populations and other primates, few of them go deeply into the real causes and mechanisms of microwear formation (Peters, 1982; Teaford, 1988; Maas, 1991; Teaford and Runestad, 1992). Understanding the etiological factors concerning microwear may help us to assess the potential of different parameters to discriminate diets (Maas, 1991).

The microwear pattern is a dynamic, age-dependent process, both cumulative and renovating (Bullington, 1991; Pérez-Pérez et al., 1994). Experimental studies in vivo, although sometimes controversial (Gordon and Walker, 1983; Kay and Covert, 1983; Teaford and Oyen, 1989a,b; Teaford and Tylenda, 1991), constitute an important source

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of information on the formation and obliteration of the microwear features. It has been proved that the addition of exogenous abrasive particles (such as grit) to the food of different mammals yields a fast increase in the appearance of scratches on the enamel.

Several studies point out that silica phytoliths probably constitute a major source of abrasion intrinsic to the food (Baker et al., 1959, 1961; Walker et al., 1978; Fine and Craig, 1981). For instance, the presence of phytoliths in grasses can contribute to the fast rate of wear in the teeth of herbivores (Baker et al., 1959).

Plants absorb the silica dissolved in the soil, along with nutrients, through the root system. The silica solidifies in the plant tissues, either intra- or extracellularly, adopting different sizes and shapes, depending on the tissue and the plant (Piperno, 1988). Certain families, like Poaceae (Kaplan et al., 1992), Cyperaceae (Ollendorf, 1992) and Equisetaceae, are especially rich in phytolith content, while in others, like Leguminosae, these particles are scarce (Jones and Handreck, 1965; Cummings, 1992). Silica phytoliths are mainly composed of SiO_2, with 5–15% H_2O and occasional trace elements, such as Mg, Ca, K, Fe, Al, and organic carbon (Rovner, 1983). Redundancy (existence of different plants with similar phytolith morphology) and multiplicity (different phytolith morphologies in the same plant) are the most important limitations in phytolith taxonomy (Piperno, 1988).

Silica is one of the few materials that can scratch the surface of tooth enamel, which is the hardest biological tissue known. Most phytoliths have diameters ranging from 2 to 100 μm (Rovner, 1983; Walker, 1981). According to Maas (1991), grit particles between 14 and 73 μm of diameter probably produce striations of about 1–5 μm width, which is the usual range of striation widths observed on the enamel surface of human teeth (Peters, 1982; Puech, 1982). Ciochon et al. (1990) have observed phytoliths of different morphologies sunk at the end of scratches in teeth of *Gigantopithecus blacki*. The authors suggested that those phytoliths produced the striations. However, postmortem effects cannot be truly ruled out, especially in fossilized specimens. It is also difficult to establish with certainty what percentage of striations can be attributed to the effect of phytoliths and other abrasives. In fact, phytoliths embedded in scratches are an extremely rare phenomenon, perhaps as few as one in every 1,000–10,000 striations (Lalueza et al., 1994). Studies of buccal microwear in several human populations have shown that vegetarians (Hindus) have significantly more striations than do predominantly meat-eating groups (such as Inuit and aborigines from Tierra del Fuego) (Lalueza and Pérez-Pérez, 1993). If phytoliths are a major cause of at least part of these striations, the tendency toward fewer striations in carnivorous groups can be explained by the absence of phytoliths in their diets. However, other hard objects, such as grit, dust accumulation (Ungar et al., 1995), ashes (which have calcium oxalate phytoliths), or simply ancient phytoliths in the soil, can also produce striations when accidentally ingested with the food (Gordon, 1982).

Armitage (1975) isolated phytoliths from the dental calculus of extinct herbivores from different historical sites in Britain, thus providing a new source of information concerning phytoliths. More recently, Middleton (1990, 1992) and Middleton and Rovner (1994) have improved methods of phytolith extraction and applied them to the study of the dental remains of cows, sheep, and pigs recovered from archaeological sites. At the moment, however, there is only one pilot study on the isolation of phytoliths on the enamel surface of human teeth (Lalueza et al., 1994), and two brief reports on the observation of phytoliths in human dental calculus (Middleton, 1993; Holt, 1993).

Dental microwear is related to age, diet, techniques of food cleaning and processing, environment, cultural habits involving the anterior dentition, and also to biomechanics. The combination of all these factors makes the study of dental microwear a complex problem that sometimes yields confused or oversimplified results. The integration of microwear data with other sources of information (e.g., phytoliths) can shed new light on paleodietary and paleoenvironmental studies. The purpose of the present study is to develop different methods for recovering

phytoliths from human dental calculus, and to compare those phytoliths with those on the enamel surface of teeth from the same individuals. The phytoliths obtained from the soil of the abdominal area of the buried individuals are also studied, and compared to the phytoliths from other soil samples inside the burials. If all results are in agreement, the study of these silica particles might yield insights into the diet of these populations.

MATERIALS AND METHODS

Archaeological sample

A necropolis from the late Roman period was excavated in Tarragona (in the northeast portion of present-day Spain) during 1993. The Roman city of Tarraco was the political and mercantile center of the province of Hispania Citerior. The recently excavated necropolis seems to belong to a larger necropolis on the western boundary of the modern city of Tarragona (Del Amo, 1979), and is dated to ca. 300–550 AD (Foguet and Vilaseca, 1995). The burials were west–east oriented, scattered over a layer of sand that constituted the ancient coastline. A minimum of 155 individuals have been excavated from this site (Lalueza y García, 1994).

Although some burials have collapsed due to earth pressure, the sample used here is from intact individual burials, with no signs of reutilization or disturbance. Thus, the bones were recovered in anatomical connection. The burials selected in this study were constructed with two empty amphora, a Roman large-handled vessel. Previously, one end of each amphora was broken in circular section. Then, both amphoras were fitted together with the body placed inside, and the fissures carefully filled with mortar. After that, the connected amphoras were deposited over the sand. Due to the closed structure of these tombs, soil intrusions from the site environment and postmortem sedimentary processes probably had a negligible effect in the selected burials, although the possibility that phytoliths from the soil of the site were already present in some preexisting soil inside the amphoras before the introduction of the bodies cannot be completely discarded.

Eleven of the adult individuals of the population (n = 107) had dental calculus, but the volume of the calculus varied significantly between individuals. This may be attributable in some cases to the existence of unilateral gross oral pathologies (Lalueza y García, 1994). Calculus is mainly formed by calcites and phosphates, and during the life of the individual acts as a trap for food debris (Hardie and Bowden, 1974), bacteria (Brothwell, 1981), and other particles, such as phytoliths. Seven individuals (numbered T42, T43, T62, T120, T122, T124, and T127) with medium degrees of dental calculus in the whole dentition, according to the classification of Brothwell (1981), and also with intact burials, were used in this study.

Sample preparation

A modification of the protocol of Middleton (1990), adapted to the characteristics of human dental calculus, was followed. The teeth were cleaned with a soft toothbrush and abundant distilled water, in order to eliminate possible soil residues and contaminating phytoliths from the surface of the dental calculus. Pieces of calculus (between 0.1 and 0.5 g) were then removed with the aid of a scalpel, and kept in sterile plastic bags. One fragment of calculus from each individual was placed directly on a stub for microscopy, and then crushed with sterile tweezers and coated with a 400 Å layer of gold. The remaining fragments of dental calculus were immersed in 20% hydrochloric acid for 6–12 hr. The liquid was evaporated overnight in a stove at 70°C. As phytoliths are resistant to the effect of the acid, the residue was mainly phytoliths. The residue was then placed on a stub and directly coated with gold.

For comparative purposes, two other potential sources of phytoliths were examined.

Enamel surfaces. One tooth was extracted from each individual, coated with gold, and observed by SEM, searching for phytoliths spatially related to striations on the enamel. The method was the same as that used in Lalueza et al. (1994), which is a modification of that of Ciochon et al. (1990). The tooth was first immersed in a 20% acetic acid solution for 15 min to remove carbonate deposits from the surface, then in 70% alcohol for 15

min to remove acid residues, and finally in distilled water for 15 min. The tooth was then dried in a stove, directly coated with a layer of gold of about 400 Å, and observed by SEM.

Burial soil. The soil from the abdominal area of several burials was processed to obtain phytoliths. The area selected was between the lumbar vertebrae and the sacrum, and corresponds to the area occupied by the viscera during the life of the individual. It was assumed that the phytoliths from the stomach were deposited around this area after the decaying of the corpse. Owing to the closed structure of the burials, few sedimentary processes are assumed to have occurred inside the burials, and accordingly contamination of soil was probably minimal. Nevertheless, as water filtration may have altered or displaced the original phytoliths from the stomach area, samples of soil from other areas inside the burials (e.g., near the feet) were also examined.

The method followed for the soil samples was based on that of Cummings (1994). The sample (50 g) was sieved in a mesh of 250 μm, in order to retain large debris, and then dissolved in 50 ml of sodium hexa-metaphosphate to separate the clays, changing the liquid every 2 hr. This process was repeated for 48 hr, and the sample was then washed in distilled water. Then, 50 ml of 40% sodium hypochlorite was added to the sample for about 12–24 hr to eliminate organic material. After this, the sample was washed in distilled water and again with sodium hexa-meta-phosphate to eliminate possibly clay particles liberated by the action of the sodium hypochlorite. The calcium carbonate was removed by washing in acetic acid, and the sample was washed with distilled water and air dried. Once the sample was dried, zinc bromide (2.3 density) was used to separated phytoliths from silt particles in a centrifuge at 750 rpm for 10 min. Finally, the sample was washed again with acetic acid and distilled water at 3,000 rpm for 5 min.

The phytoliths obtained were first observed with a light microscope (Olympus BH-2) at 400× magnification. The final identification was made by means of scanning electron microscopy (SEM).

Isolation and identification of phytoliths

The samples were observed in a scanning electron microscope (Cambridge Stereoscan s-120), which allows a resolution power of 6 nm, magnification from 9× to 200,000×, and an accelerating voltage from 1 to 30 kV. The SEM was operated at different magnifications and 15 kV accelerating voltage in secondary electron mode. The siliceous nature of the phytoliths was verified by X-ray microanalysis while in the microscope. Both the enamel and the calculus samples were observed at 400× magnification. Once a phytolith was located, the magnification was increased to 3,000–5,000×, depending on the size of the particle, and the phytolith was photographed. The samples were tilted at different angles to obtain a three-dimensional impression that could facilitate the identification of the phytoliths. Later, the phytoliths were classified by comparing their micrographs with a large reference sample of present-day plants from the Mediterranean area. The comparative collection includes over 1,000 species of plants from 70 taxa, 140 of them corresponding to edible vegetables (Figs. 1, 2). Information from classic Latin writers about feeding behavior during the Roman period was also taken into consideration (André, 1961).

The main objective in identifying phytoliths is to correlate the size and shape of the particle with the location of the cell of origin in the plant tissue (Piperno, 1988). The classification was based on morphological characteristics, following the systematics of Twiss (1992), Mulholland and Rapp (1992), and Pearsall and Dinan (1992). Measurements of size of the particles were made from the micrographs with the aid of an image analyzer system (IBAS II).

RESULTS

Phytoliths from the enamel

Phytoliths from different cereal plants (Table 1) were discovered scattered all along the enamel, especially on the lingual and buccal surfaces. Presumably tooth-to-tooth contact prevents the preservation of sili-

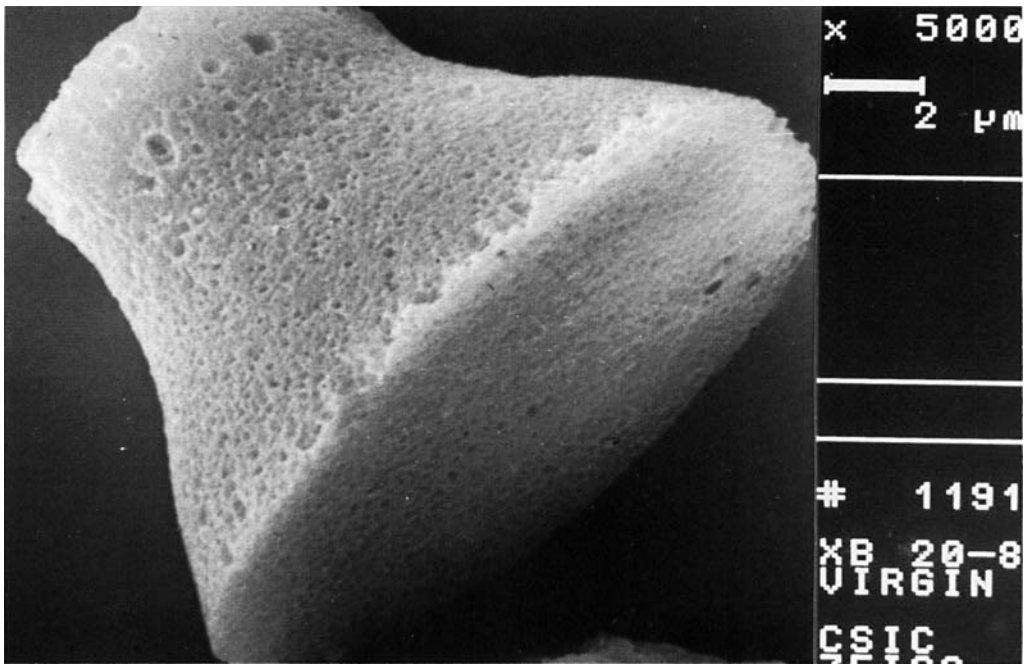

Fig. 1. Silica phytolith (festucoid shape, tower type) from a modern Poaceae plant (×5,000 magnification), belonging to the reference sample.

ceous particles on occlusal wear facets. On the buccal surface, the microwear seems to have a slower turnover than on the occlusal surface (Pérez-Pérez, et al., 1994); thus, phytoliths there may remain undisturbed for a long time. Only particles that were at the end of a scratch were considered. As there was no taphonomical process at this site that could have sunk a phytolith into the enamel, this ensures that these phytoliths were ingested during the life of the individual. The method is more restrictive than that followed by Ciochon et al. (1990), who considered phytoliths found sunk into the enamel even if they were not associated with scratches. Including these phytoliths would increase the sample size significantly. The lack of scratches and scratch-related phytoliths observed on teeth emerging shortly before the death of the individual is also indirect evidence of the absence of significant postmortem processes affecting the enamel surface.

In Figures 3 and 4, two phytoliths can be observed on the buccal enamel of right lower first incisors of two different individuals. Both were selected for their clear relationship with a scratch, although only one was classified as a Poaceae phytolith. Normal scratches related to diet are usually different from the sinuous scratch observed in Figure 3; however, similar striations have been described on occasion (Puech et al., 1980).

Phytoliths in the dental calculus

Several phytoliths were observed embedded in the dental calculus, some of them largely covered by carbonate deposits, thus hindering their identification (Table 1). The physical inclusion of the silica particles in the matrix of the calculus is an indirect evidence for the contemporaneity of both structures, as the mineralization of dental plaque occurs in the presence of saliva (MacPhee and Cowley, 1975); thus, no postmortem remodeling of dental calculus has been noticed (Middleton and Rovner, 1994). In Figures 5, 6, and 7, different phytoliths included in the dental calculus are presented, all belonging to the Poaceae family.

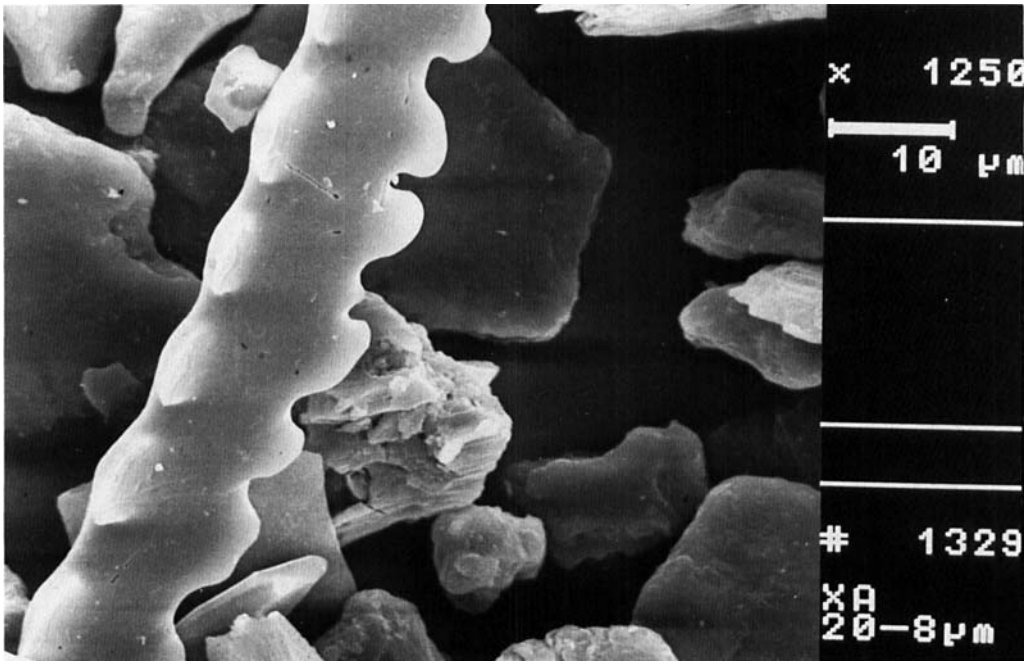

Fig. 2. Silica phytolith (polylobate type) from a modern Poaceae plant (×1,250 magnification), belonging to the reference sample.

In the samples treated with hydrochloric acid, some phytoliths appeared mixed with undigested calculus remains. Some of these remains are morphologically similar to silica phytoliths (Fig. 8). However, the true phytoliths were easily identified by X-ray microanalysis due to their siliceous composition. In this case, measures of precaution against possible environmental contaminations must be adopted (e.g., storing the digested samples in sterile plastic bags until the SEM session).

Phytoliths from the burial soil

Soil samples of 50 g from the abdominal area were obtained during the excavation and kept in sterile plastic bags. Unfortunately, coprolites attributable to hardened postmortem excrements, which are a source of phytoliths directly related to the late meals (Cummings, 1993), were not present in our sample. Control samples from the peripheral area of the burials were obtained from 50 g of soil adhering to the bones of the foot.

In the abdominal samples, phytoliths belonging to four families, Leguminosae (leguminous), Poaceae (gramineous), Chenopodiaceae (chenopods), and Cyperaceae (sedges), were found (Table 1), although the Poaceae specimens were the most abundant (69.7%). A large number of calcium oxalate crystals associated to multicellular aggregates (Chang and Beevers, 1968; Al-Rais et al., 1971; Doaigey, 1991) belonging to the Chenopodiaceae family were also found in one individual. Although calcium oxalate crystals are rarely diagnostic to family, some of them are compatible with edible species abundant in the area, such as *Beta vulgaris* (beet), *Chenopium bonushenricus* (wild spinach), *Spinach* sp. (spinach), and *Vicia faba* (bean). However, attribution to other vegetables cannot be ruled out at present, and thus this identification is only tentative.

Although it is not possible to demonstrate that all the phytoliths come from the stomach contents of the individual, the results are in agreement with the human diet in the

TABLE 1. Phytoliths identified, following the classification of Mulholland and Rapp (1992) and Pearsall and Dinan (1992)

Sample	Total phytoliths	Poaceae phytoliths	Undetermined	Festucoid shape		Larger grass cells		
				Round	Square	Bulliform	Long cell	Trichoma
T42 left M^1 (fragmented calculus)	5	5	—	—	4	—	—	1
T43 right I_1 (fragmented calculus)	9	2	7	1	—	—	1	—
T43 right I_1 (enamel surface)	5	1	4	—	—	1	—	—
T62 right I_1 (fragmented calculus)	5	—	5	—	—	—	—	—
T62 right I_1 (digested calculus)	10	6	4	—	—	—	3	3
T62 right I_1 (enamel surface)	2	—	1	—	—	1	—	—
T120 right I_1 (enamel surface)	1	—	1	—	—	—	—	—
T120 right I_1 (digested calculus)	—	—	—	—	—	—	—	—
T122 right I_1 (fragmented calculus)	2	—	—	1	—	—	—	1
T124 right I_1 (digested calculus)	2	—	1	—	—	—	1	—
T124 left M_3 (enamel surface)	3	—	1	—	1	—	1	—
T124 left M_3 (digested calculus)	2	—	1	1	—	—	—	—
T127 right Pm_3 (enamel surface)	1	1	—	—	1	—	—	—
T127 right Pm_3 (digested calculus)	—	—	—	—	—	—	—	—

	Total phytoliths	Poaceae phytoliths		Undetermined	Chenopodiaceae	Leguminosae	Cyperaceae
		Festucoid	Long cells				
Burial soil samples							
T42 (abdominal area)	—	—	—	—	—	—	—
T43 (abdominal area)	—	—	—	—	—	—	—
T62 (abdominal area)	86	12	7	—	43	24	—
T120 (abdominal area)	146	52	82	12	—	—	—
T122 (abdominal area)	72	23	12	37	—	—	—
T124 (abdominal area)	80	32	21	3	—	—	24
T127 (abdominal area)	127	83	32	12	—	—	—
Peripheral samples							
T42 (foot bones area)	—	—	—	—	—	—	—
T43 (foot bones area)	—	—	—	—	—	—	—
T62 (foot bones area)	25	3	21	1	—	—	—
T120 (foot bones area)	36	12	21	3	—	—	—
T122 (foot bones area)	18	6	10	2	—	—	—
T124 (foot bones area)	18	—	12	2	—	—	4
T127 (foot bones area)	—	—	—	—	—	—	—

Mediterranean area. Extensive contamination by present leguminous phytoliths from the adjacent soil is unexpected, owing to the early inclusion of this necropolis within the urban area of the medieval city. To test the hypothesis of environmental contamination, we have analyzed the phytoliths of the soil from a peripheral area of the burial (Table 1). In four cases phytoliths from Poaceae were found in both samples, while in one case they were found only in the abdominal samples. Chenopodiaceae and Leguminosae phytoliths were present only in abdominal samples. In absolute values, phytoliths were more abundant in the abdominal samples than in the peripheral samples. Differences are statistically significant, by applying a Wilcoxon paired test ($t = -2.0226$, $P < 0.05$). However, soil samples from two specimens (T42 and T43) do not yield any silica particle in the analysis. A possible interpretation is that the absence of silica in these samples could indicate an absence of stomach contents immediately before death.

DISCUSSION

All particles isolated gave positive results in the X-ray microanalysis, indicating the massive presence of silica in their composition. However, although silica phytoliths could be undoubtedly identified, most of them were difficult to classify down to the family level ("undetermined" in Table 1), due not only to the inherent limitations of phytolith taxonomy, but also to postmortem modifications in the morphology of some of these particles. The phytoliths on the enamel can be physically abraded by the action of the food ingested after the fixation of the particle

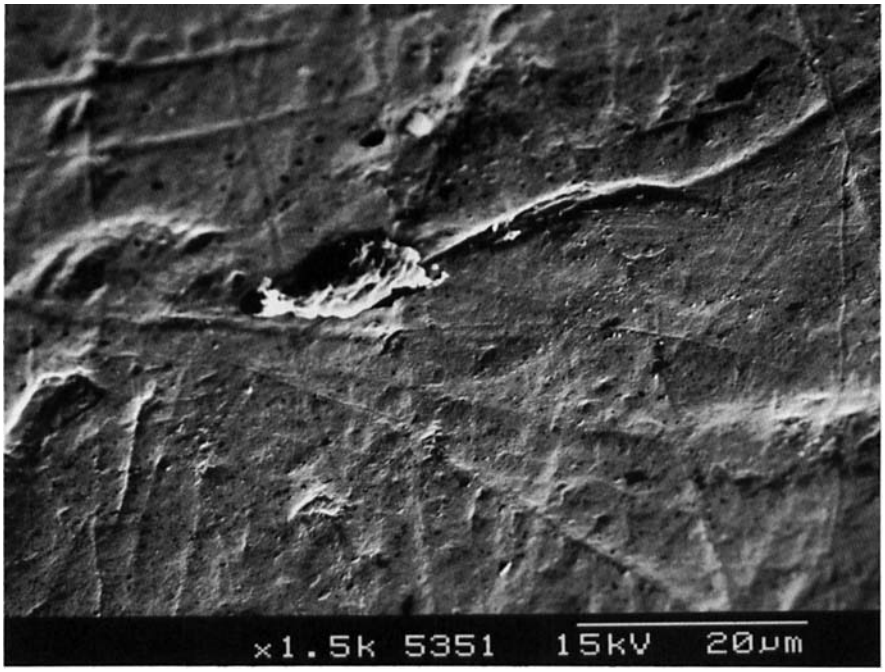

Fig. 3. Silica particle of undetermined morphology on the buccal enamel of a right I_1 (T43). The phytolith is clearly associated with a sinuous scratch from the right (×1,500 magnification).

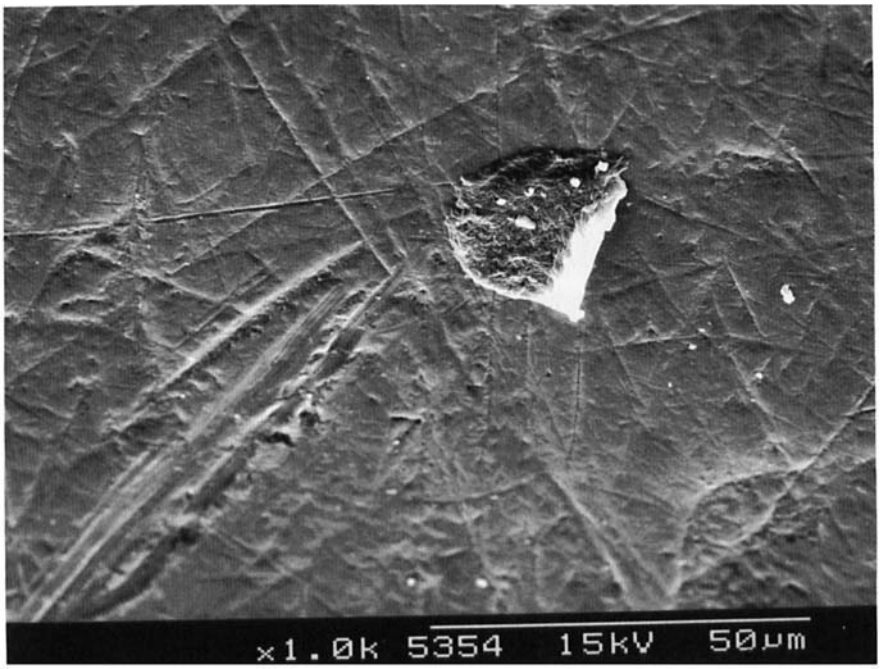

Fig. 4. Bulliform phytolith on buccal enamel of a right I_1 (T62), which seems to be related to several scratches from the left (×1,000 magnification).

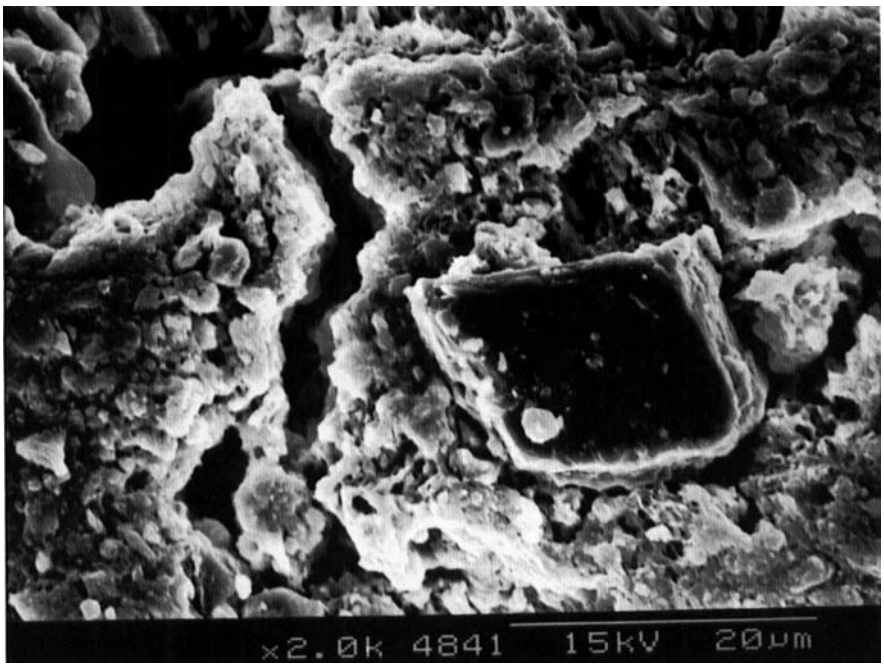

Fig. 5. Square phytolith of the T42 left M^1 embedded in a fragmented piece of buccal calculus (×2,000 magnification).

to the tooth. Moreover, different factors (such as pH, temperature, or chemicals of the soil) may modify the solubility of phytoliths, altering their characteristics postmortem.

It is difficult to understand how phytoliths became bonded to the buccal or lingual enamel. It may be related both to the physical consistency and size of the bolus together with the pressure of the cheek muscles over the buccal surface of the dentition (or to the pressure of the tongue over the lingual surface). The possibility that some phytoliths could correspond to ancient particles from the soil adhering to the food's surface cannot be excluded at present. However, this question could be elucidated in the future with experimental studies.

The fact that phytoliths of only a particular morphology and size were encountered on the enamel and dental calculus can at least be suggestive evidence in support of the reliability of this kind of study. The presence of cereal phytoliths is concordant with the human diet during the Roman period in Tarragona. Also, no phytoliths were found that could be excluded from a human diet using ethnobotanical criteria (e.g., phytoliths from Equisetales, a vegetable group in which they are particularly abundant). This may be additional evidence for the lack of environmental contamination. On the other hand, the size and shape of the silica bodies may be related to the capacity of the particle to become included into the dental calculus or sunk and preserved into the enamel. In this sense, sharp morphologies of phytoliths with spines seem to be overrepresented in our sample. For instance, no examples of panicoid morphologies were found, although these are well represented in the Poaceae.

The phytoliths isolated from the abdominal area shed a different light on the possible diet of this Roman population. In those samples, most phytoliths belong to the Poaceae family, although significant amounts of particles from other families (Chenopodiaceae, Leguminosae and Cyperaceae) are also present. This discrepancy can have different interpretations. Perhaps the morphology of

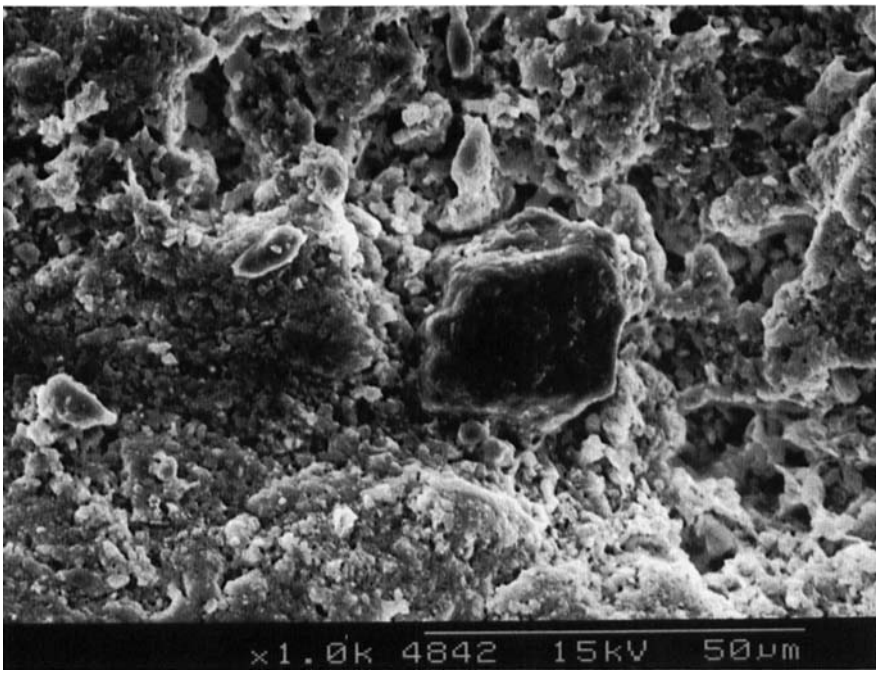

Fig. 6. Square phytolith in the calculus of the T42 left M^1. The phytolith is covered by carbonates and fixed to the calculus matrix (×1,000 magnification).

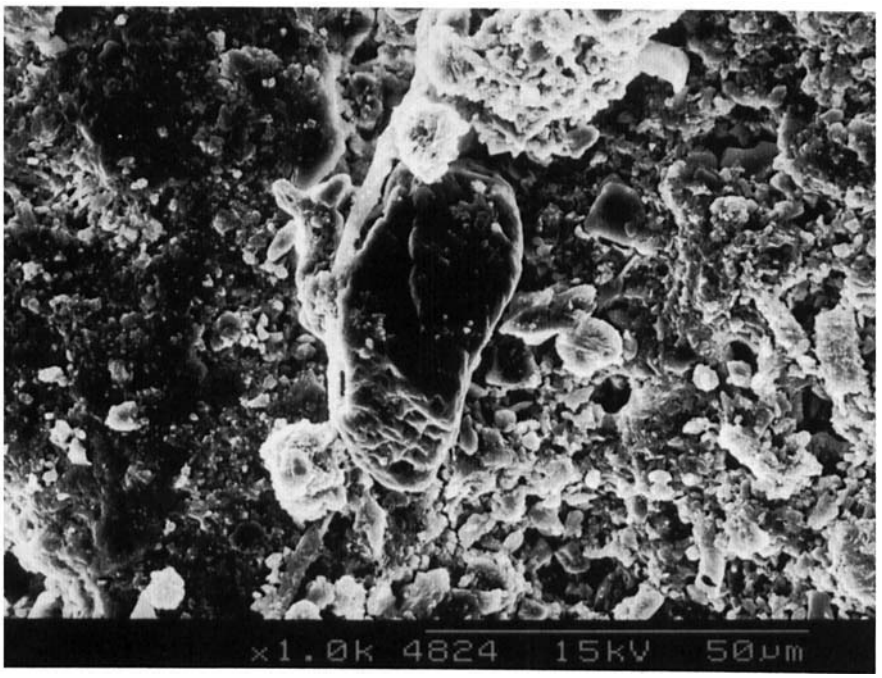

Fig. 7. Round phytolith in the calculus of the T43 right I_1 (×1,000 magnification).

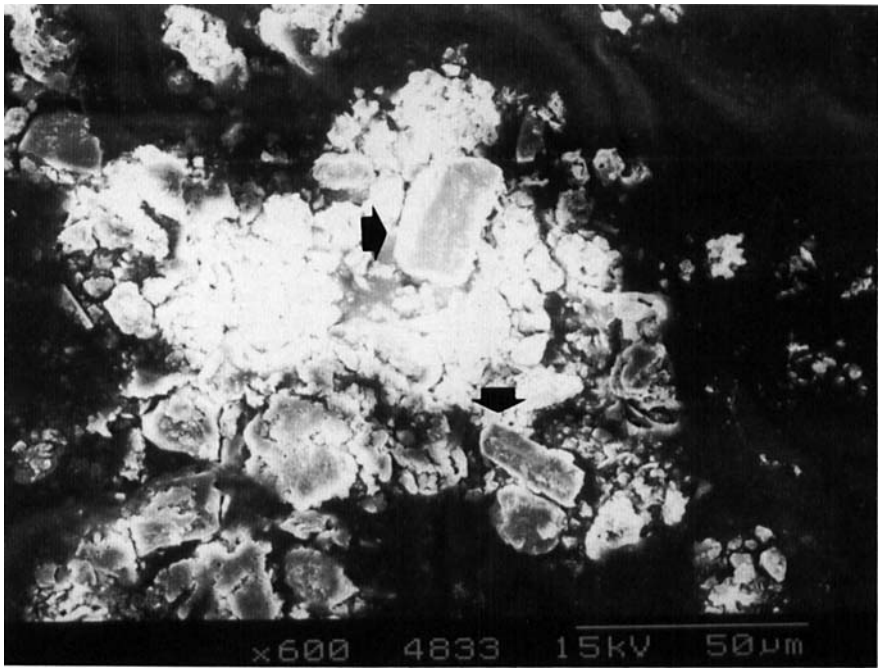

Fig. 8. Calculus sample of T62 right I_1, after treatment with hydrochloric acid. Two phytoliths (arrows) can be distinguished between the undigested calculus remains (×600 magnification).

certain phytoliths, once again, precludes their inclusion on the calculus and the enamel surface. For instance, the low amounts of Leguminosae phytoliths may mean that they are less likely to be attached to teeth. Likewise, the presence of contaminating phytoliths from some kind of preexisting soil cannot be ruled out. Also, the exact way in which the bodies were introduced into the amphora burials is unknown; the corpses probably laid over the sand during the construction of the burial. In addition, water filtrations may have mixed phytoliths within the tomb. Obviously, further research about the taphonomy of phytoliths is needed.

CONCLUSIONS

Analysis of phytoliths can yield insights into a number of topics, including paleoenvironmental reconstruction and the selection of cereals during the adoption of agriculture. However, the more promising area for phytolith research involves dietary reconstruction for past human populations. Such an approach is admittedly not without its limitations. First, phytoliths can tell us only about vegetable intake; second, only vegetables with phytoliths can be represented in this type of study; and third, not all types of phytoliths seem to be equally represented in the enamel surface or in dental calculus. Another factor to consider is the poor taxonomic power of phytolith morphologies, which do not usually allow a taxonomic classification to the species level. The main limitation of such studies, however, depends basically on our ignorance of phytolith morphologies in modern vegetation, especially those from the Mediterranean area. Some of these limitations can be avoided by considering complementary data (archaeological, ecological, ethnobotanical, and historical) from the site. Moreover, despite these limitations, the potential exists to shed new light on past human diets, once we have better knowledge of modern phytoliths.

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